Research Results in Pharmacology

Research Results in Pharmacology 12(1): 40–47
UDC 616.13-004.6:57.087.3
DOI 10.18413/rrpharmacology.12.1078

Research Article

Morphometric visualization, analysis, and assessment of atherosclerotic lesions in an apolipoprotein E–deficient mouse model (ApoE−/−)

Petr R. Lebedev[1], Natalya V. Syrykh[1], Mikhail V. Pokrovskii[1]

1 Belgorod State National Research University; 85 Pobedy St., Belgorod 308015 Russia

Corresponding author: Petr R. Lebedev (artkelt98@yandex.ru)

Academic editor: Oleg Gudyrev ♦ **Received** 13 January 2026 ♦ **Accepted** 01 March 2026 ♦ **Published** 19 March 2026

Citation: Lebedev PR, Syrykh NV, Pokrovskii MV (2026) Morphometric visualization, analysis, and assessment of atherosclerotic lesions in an apolipoprotein E–deficient mouse model (ApoE−/−). Research Results in Pharmacology 12(1): 40–47. https://doi.org/10.18413/rrpharmacology.12.1078

Abstract

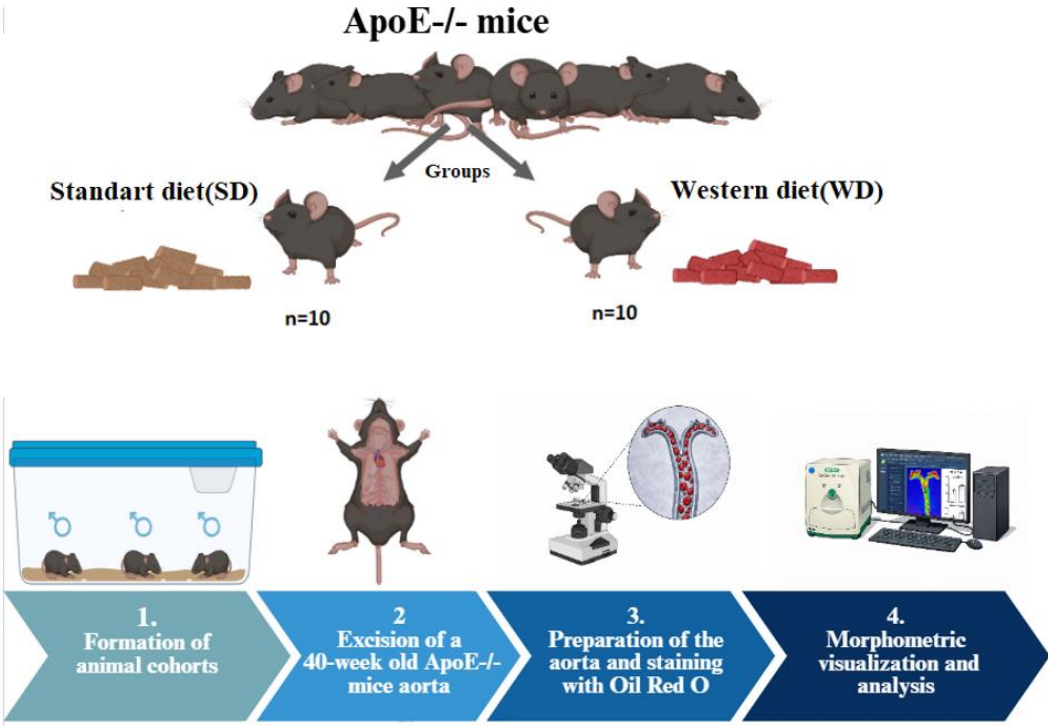
Introduction: The improvement of diagnostic methods in atherosclerosis is aimed at accurately determining the stage of the pathological process and eliminating subjective interpretation of data. Implementation of this approach increases the reliability of comparative assessment of the effectiveness of therapeutic and interventional strategies and reduces statistical variability within experimental studies.

Materials and Methods: The study was performed on 20 male ApoE−/− mice (40 weeks old) divided into groups receiving a standard diet or a Western diet. After anesthesia, PBS perfusion was performed; the heart with the aorta was excised and fixed in 10% formalin; the aorta was cleaned and stained with Oil Red O. The aorta was longitudinally opened, photographed (GelDoc), and quantitatively analyzed using a Python script. Data are presented as mean ± SD; statistical significance was assessed using Student's t-test at $p < 0.05$.

Results and Discussion: Thus, the use of the Oil Red O staining method in combination with automated image analysis in Python enables a transition from subjective assessment to precise quantitative measurement of the area of atherosclerotic lesions. This approach increases the reliability of screening therapeutic interventions in experimental models and enhances the statistical power of the study.

Conclusion: In this study, an original approach to morphometric visualization, analysis, and quantitative assessment of atherosclerotic lesions of the vascular wall in an apolipoprotein E–deficient mouse model (ApoE−/−) is proposed and validated for the first time.

Graphical Abstract



Keywords

atherosclerosis, aorta, ApoE−/− model, knockout mice, apolipoprotein E deficiency, morphometric analysis

Introduction

Atherosclerosis is a chronic progressive disease of the arterial system characterized by the accumulation of lipids, inflammatory cells, and extracellular matrix components in the vascular wall, leading to the formation of atherosclerotic plaques, the narrowing of the arterial lumen, and impaired blood flow (Pedro-Botet et al. 2020). These processes underlie coronary heart disease (Stone et al. 2023), stroke (Evans et al. 2024), and other cardiovascular complications, making atherosclerosis one of the leading causes of mortality worldwide. It is driven by complex pathophysiological mechanisms, including endothelial dysfunction (Shcheblykin et al. 2022), dysregulation of lipid metabolism (Cao et al. 2020), and proliferation of vascular smooth muscle cells (Miano et al. 2021), and is currently regarded as a systemic inflammatory disease of the vasculature.

Experimental models are widely used to study the mechanisms of atherosclerosis and to evaluate the efficacy of therapeutic strategies. One of the most common genetic models is the apolipoprotein E knockout mouse (ApoE−/−). ApoE deficiency leads to pronounced hypercholesterolemia and spontaneous development of atherosclerotic plaques even under a standard diet, allowing modeling key stages of disease development similar to human lesions (Noonan et al. 2025).

Existing methods for assessing atherosclerosis include traditional histology followed by manual or automated lesion measurement (Holmberg et al. 2021), the use of specialized tomographic systems (Stadelmann et al. 2021), as well as analysis of gross specimens using dedicated image analysis software. Morphometric visualization and quantitative assessment of atherosclerotic lesions are fundamental approaches for determining plaque area and severity in experimental models. Morphometric analysis of lesion size, distribution, and structure is considered a standard in experimental atherosclerosis diagnostics, providing numerical parameters of vascular damage (Mohanta et al. 2016). These data are essential for comparing the effects of genetic modifications, dietary interventions, and pharmacological treatments.

However, manual or partially automated processing can lead to substantial dependence of results on the investigator's experience, segmentation threshold selection, and tissue preparation quality, thereby reducing reproducibility and objectivity. Many approaches require extensive manual labor and significant operator time to process large image datasets, limiting their efficiency in large-scale experiments (He et al. 2024).

Aim of the study: To evaluate a novel method for morphometric visualization, analysis, and assessment of atherosclerotic lesions in ApoE-deficient mice.

Materials and Methods

Animals

The study was approved by the Institutional Animal Care and Use Committee of Belgorod State National Research University (expert opinion No. 01-08и/25 dated 18.08.2025). ApoE−/− mice were housed under SPF conditions in the animal facility of Belgorod State National Research University under controlled light conditions (12 h light/12 h dark) at a temperature of +22 to +26 °C, with free access to food and water. All procedures complied with the ethical principles of laboratory animal care in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 170) and Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes.

Animal groups

The study was conducted on ApoE−/− laboratory mice, a well-established model of experimental atherosclerosis. A total of 20 male ApoE−/− mice were randomly assigned to two equal groups (n = 10 each).

The first group was maintained on a standard laboratory diet (SD) throughout the observation period. The second group received a high-fat Western diet (WD), initiated at 8 weeks of age to induce and accelerate the development of atherosclerotic lesions.

Study design

After intraperitoneal anesthesia with zolazepam (2.5 mg/100 g) and xylazine (2 mg/100 g), animals were perfused with cold PBS (30 mL, 4 °C). The heart with the aorta was excised and fixed in 10% neutral buffered formalin for 12–24 h. The aorta was cleaned of perivascular tissue, defatted in 78% methanol, and stained with Oil Red O to visualize lipid deposits. The vessel was opened longitudinally, flattened endothelium-side up, and photographed using a Gel Doc EZ Imager system.

Morphometric analysis was performed on TIFF images using ImageJ and an automated Python-based algorithm (NumPy, OpenCV, Pillow).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Quantitative data on atherosclerotic lesion area followed a normal distribution. Intergroup comparisons were performed using an unpaired Student's t-test. Data are presented as mean ± standard deviation; $p < 0.05$ was considered statistically significant.

Results and Discussion

Experimental protocol for aorta harvesting, processing, and morphometric analysis

Protocol for aorta and heart harvesting

To obtain the aorta in a standardized and atraumatic manner while preserving the anatomical integrity of the vascular bed, proper euthanasia and perfusion are required to ensure effective removal of blood from the vessels. This step is critically important for subsequent tissue fixation and accurate assessment of lipid deposition. The aorta harvesting procedure used in this study is described below;

1. Prior to necropsy, prepare all required materials: clean surgical instruments (straight forceps, dissection scissors, ophthalmic scissors, dental probe), a dissection board with pins, paper towels, chilled PBS solution, containers with 10% neutral buffered formalin, and a Class B biohazard waste bag.
2. Subject the mouse to anesthesia followed by euthanasia using CO_2 inhalation, cervical dislocation, or decapitation.

3. Place the mouse in a supine position and secure the forelimbs and hindlimbs with pins on a dissection board covered with 3–4 layers of paper towels.
4. Moisten the fur with a small amount of 70% ethanol to minimize hair contamination.
5. Using scissors, perform a midline incision through the skin, subcutaneous tissue, and peritoneum from the abdominal cavity to the thoracic region.
6. Secure the skin and subcutaneous tissue to the platform with pins for improved access.
7. Open the mediastinum and cut the ribs laterally to the sternum. Lift the sternum with forceps by grasping the xiphoid process. Transect the diaphragm to facilitate access to the heart and aorta.
8. Create an opening in the right atrium. Perfuse the vascular system via apical puncture of the left ventricle using chilled PBS (4 °C), with a total volume of 30 mL at a flow rate of 3–5 mL/min.
9. To access the aorta, remove organs and tissues located in close proximity to the vessel.
10. Grasp the iliac portion of the aorta with straight forceps together with adjacent tissues and carefully dissect the connective tissue between the aorta and the abdominal wall, proceeding cranially toward the aortic arch.
11. Directing the tips of the scissors cranially (toward the head), transect the thymus and the arteries contained within it to allow free extraction of the aorta.
12. Place the harvested aorta and heart into a container with 10% neutral buffered formalin at 4°C overnight or for up to 24 h for fixation (tissue-to-fixative ratio 1:20).
13. The aorta can subsequently be processed for Oil Red O (ORO) staining.

Fixation, aorta preparation, and Oil Red O staining

This stage is aimed at preserving the morphological structure of the aorta and enabling selective visualization of lipid inclusions within the vascular intima. Tissue fixation stabilizes structural components, while sequential methanol treatment and Oil Red O staining allow specific detection of neutral lipids and atherosclerotic lesions. Careful removal of surrounding tissue and longitudinal opening of the aorta ensure proper flattening of the vessel and uniform presentation of the entire surface for analysis. A detailed step-by-step description of the procedure is provided below:

1. Place the aorta and heart in a plastic Petri dish containing PBS, ensuring that the tissues remain constantly hydrated.
2. Carefully dissect the thymus using ophthalmic scissors, a dental probe, and straight forceps under a stereomicroscope equipped with a top-mounted light source at 30–40× magnification. Extreme care should be taken to avoid damaging the aorta during thymus removal.
3. Remove perivascular connective tissue and adipose tissue surrounding the aorta and major arterial branches: the brachiocephalic artery, left common carotid artery, and left subclavian artery of the thoracic aortic arch, as well as the common iliac arteries of the abdominal aorta. Gently retract adventitial fat using fine forceps, avoiding excessive manipulation of the vessel.
4. Transfer the cleaned aorta into a 1.5 mL microcentrifuge tube.
5. Add 1 mL of 78% methanol and place the tube on a low-speed shaker for 5 min.
6. Replace the methanol solution and repeat the previous step twice.
7. Remove the methanol and add 1 mL of freshly prepared Oil Red O solution: dissolve 0.07 g of Oil Red O in 25 mL of 100% methanol, then add 10 mL of 1 M NaOH.
8. Incubate the tube with the ORO solution on a low-speed shaker for 50–60 min.
9. Transfer the aorta to a clean tube and wash twice with 1 mL of 78% methanol for 5 min each on a low-speed shaker.
10. Remove the methanol and refill the tube with 1 mL PBS. If necessary, the aortas may be stored at 4 °C at this stage.
11. Using fine forceps, place the stained aorta into a Petri dish.
12. Under a stereomicroscope, carefully remove any small fragments of stained residual adventitial fat that may remain attached to the outer surface of the aorta.
13. Perform a longitudinal incision of the aorta: insert the tip of the ophthalmic scissors into the vessel lumen and cut along the outer curvature of the aortic arch to the end of the bend. Then cut along the inner curvature of the arch, extending the incision to the bifurcation of the iliac arteries. Cut the iliac arteries.
14. Mount the aorta with the endothelial surface facing upward on a substrate, carefully spreading the vessel and its branches.

Morphometric visualization and analysis

This section is required for the quantitative assessment of atherosclerotic lesion area along the entire length of the aorta. Standardized macroscopic imaging provides reproducible image acquisition conditions, eliminating variability in illumination and scale. Subsequent digital

image processing and automated analysis enable objective and reproducible evaluation of vascular wall involvement, thereby minimizing investigator-dependent bias.

1. Visualization of the preparations was performed using a GelDoc imaging system (Bio-Rad, USA) under transmitted and reflected white light modes.
2. In this protocol, the GelDoc system was used as a highly standardized digital macroimaging platform, providing uniform illumination, fixed sample positioning, and reproducible exposure parameters. This ensured acquisition of high-resolution images of the entire aortic surface with minimal geometric distortion, which is critical for subsequent morphometric analysis of lesion area.
3. The acquired images were saved in uncompressed TIFF format and processed using ImageJ software (NIH, USA). To enhance contrast between Oil Red O–positive regions and unaffected intima, pseudocolor transformation was applied using the "Pseudo color" lookup table. Pseudocoloring improved signal intensity differentiation and facilitated automated detection of atherosclerotic plaques.
4. Further analysis was performed in Python version 3.9 using the NumPy, OpenCV, and Pillow libraries. The complete analysis code with a detailed description of all processing steps is provided in Supplement 1.
5. Image analysis was conducted according to a standardized protocol to ensure reproducibility of results during repeated processing. Final output data were automatically saved as visual materials, enabling their subsequent use in statistical analysis.

Assessment of atherosclerotic esions in ApoE$^{-/-}$ mice

Based on image segmentation, binary masks were generated for atherosclerotic lesions, intact vessel wall regions, and the total vessel area. Lesion area was calculated by pixel counting, yielding both absolute values and relative values expressed as a percentage of the total vessel surface. For visual validation, overlay images were generated, in which atherosclerotic lesions were shown in red and intact vessel wall regions – in green (Fig. 1).

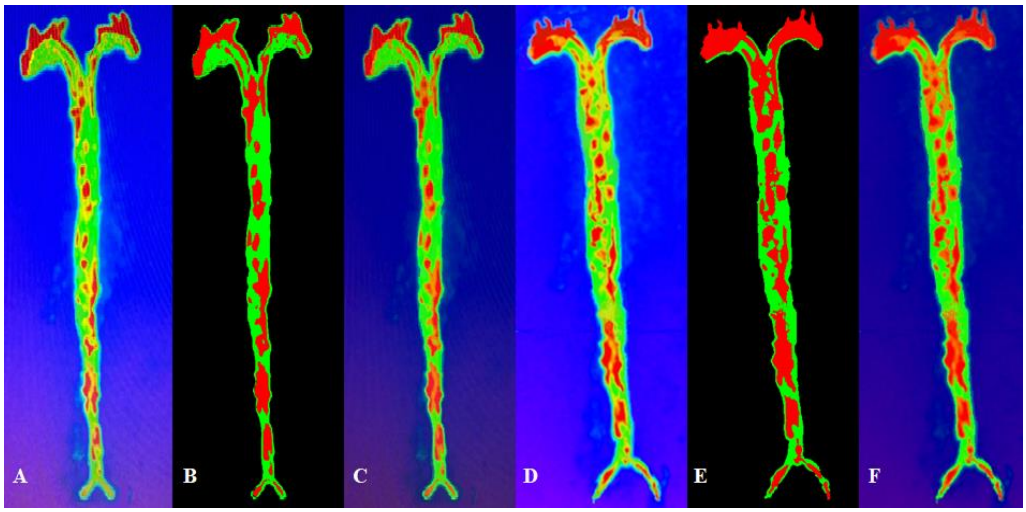


Figure 1. Binary masks of affected and intact vascular regions. ***Note:*** **A-C** – aorta of an ApoE$^{-/-}$ mouse maintained on a standard diet; **D-F** – aorta of an ApoE$^{-/-}$ mouse maintained on a Western high-fat diet. Paired images were processed using identical analytical procedures: **A** and **D** – binary masks of the aortic images; **B** and **E** – segmentation of the intima and atherosclerotic plaques; **C** and **F** – overlay of the plaque mask on the original image.

Figure 2 presents the quantitative assessment of atherosclerotic lesion area in ApoE$^{-/-}$ mice fed a standard diet (SD) or a Western diet (WD). It was found that in the WD group, the aortic atherosclerotic lesion area at week 40 was significantly higher (53.23 ± 3.72%) compared with that in the SD group (41.99 ± 3.54%). The difference between mean values (WD – SD) amounted to 11.23 ± 1.62%. Statistical analysis using an unpaired t-test revealed a highly significant difference between the groups ($t = 6.92$; $df = 18$; $p < 0.0001$).

The obtained results demonstrate that long-term consumption of a Western diet leads to a pronounced exacerbation of atherosclerotic vascular lesions in ApoE$^{-/-}$ mice compared with a standard diet. An increase in plaque area of more than 11% after 40 weeks indicates a strong pro-atherogenic effect of a high-fat diet, which is consistent with current concepts regarding the role of dietary factors in the progression of atherosclerosis (Getz and Reardon 2016; Libby et al. 2019).

At the same time, it was shown that even under standard diet conditions, ApoE-deficient mice develop statistically significant and morphologically pronounced atherosclerotic changes in the vascular wall. This finding highlights the high sensitivity and informativeness of the ApoE$^{-/-}$

model and supports its use as an adequate model for studying spontaneous atherogenesis without the need for additional dietary or pharmacological stimulation (Hinder et al. 2013). Thus, genetically determined disturbances in lipid metabolism in this animal line are sufficient to induce significant atherosclerotic lesions, whereas a Western diet acts as a powerful factor accelerating disease progression.

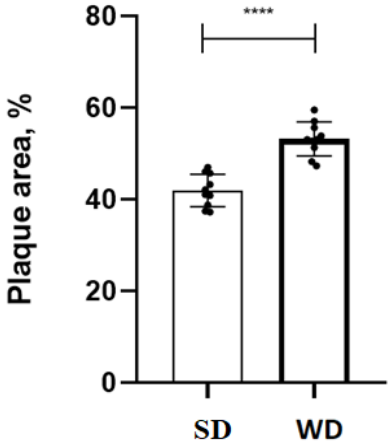


Figure 2. Quantitative assessment of atherosclerotic lesion area in ApoE$^{-/-}$ mice fed a standard (SD) or Western (WD) diet. ***Note:*** **** p-value < 0.0001.

Of particular importance in the present study is the application of a morphometric visualization, analysis, and quantitative assessment approach for atherosclerotic lesions on macroscopic aortic preparations. The employed methodology (en-face analysis combined with digital image processing) allows for objective and reproducible determination of plaque area and spatial distribution across the entire vessel surface, minimizing investigator bias and inter-operator variability (Daugherty et al. 2017; Chen et al. 2022). Key advantages of this approach include high accuracy, the possibility of automated or semi-automated analysis, standardization, and comparability of results across different experimental series.

The use of digital morphometry provides high sensitivity for detecting even moderate differences between experimental groups, which is particularly important for evaluating the effects of diet, pharmacological interventions, and genetic modifications on the extent of atherosclerotic lesions (Sheng et al. 2024).

Taken together, the obtained data confirm the suitability of the ApoE$^{-/-}$ model in combination with modern morphometric visualization techniques as an effective tool for fundamental and applied studies of atherosclerosis pathogenesis, as well as for the preclinical evaluation of novel therapeutic and preventive strategies.

Conclusion

In the present study, an original approach to morphometric visualization, analysis, and quantitative assessment of atherosclerotic lesions of the vascular wall was proposed and validated for the first time using an experimental model of apolipoprotein E–deficient mice (ApoE$^{-/-}$). The results demonstrate that this novel morphometric visualization method substantially expands the analytical capabilities of preclinical atherosclerosis research, enhances data comparability between experimental groups, and reduces subjectivity in data interpretation.

Thus, the developed method represents a new and previously undescribed tool for atherosclerosis research and makes a significant contribution to the advancement of experimental vascular morphology.

Additional Information

Conflict of interest
The authors declare the absence of a conflict of interests.

Funding
This work was supported by the Ministry of Science and Higher Education of the Russian Federation, agreement No. FZWG-2026-0002.

Ethics statement
The study was approved by the Animal Ethics Committee of Belgorod State National Research University (BelSU), approval No. 01-08i/25 dated 18 August 2025.

Data availability
All of the data that support the findings of this study are available in the main text.

References

- Cao Q, Zhao J, Xing M, Xiao H, Zhang Q, Liang H, Ji A, Song S (2020) Current research landscape of marine-derived anti-atherosclerotic substances. Marine Drugs 18(9): 440. https://doi.org/10.3390/md18090440 [PubMed] [PMC]
- Chen H, Howatt DA, Franklin MK, Amioka N, Sawada H, Daugherty A, Lu HS (2022) A mini-review on quantification of atherosclerosis in hypercholesterolemic mice. Global Translational Medicine 1(1): 72. https://doi.org/10.36922/gtm.v1i1.76 [PubMed] [PMC]
- Daugherty A, Tall AR, Daemen MJAP, Falk E, Fisher EA, García-Cardeña G, Lusis AJ, Owens AP 3rd, Rosenfeld ME, Virmani R; American Heart Association Council on Arteriosclerosis, Thrombosis and Vascular Biology; and Council on Basic Cardiovascular Sciences (2017) Recommendation on design, execution, and reporting of animal atherosclerosis studies. Arteriosclerosis, Thrombosis and Vascular Biology 37(9): e131–e157. https://doi.org/10.1161/ATV.0000000000000062 [PubMed]
- Evans NR, Bhakta S, Chowdhury MM, Markus H, Warburton E (2024) Management of carotid atherosclerosis in stroke. Practical Neurology 24(5): 382–386. https://doi.org/10.1136/pn-2023-003918 [PubMed]
- Getz GS, Reardon CA (2016) Diet and murine atherosclerosis. Arteriosclerosis, Thrombosis and Vascular Biology 36(7): 1311–1318. https://doi.org/10.1161/01.ATV.0000201071.49029.17 [PubMed] [PMC]
- He L, Chen Q, Wang L, Pu Y, Huang J, Cheng CK, Luo JY, Kang L, Lin X, Xiang L, Fang L, He B, Xia Y, Lui KO, Pan Y, Liu J, Zhang CL, Huang Y (2024) Activation of Nrf2 inhibits atherosclerosis in ApoE−/− mice through suppressing endothelial cell inflammation and lipid peroxidation. Redox Biology 74: 103229. https://doi.org/10.1016/j.redox.2024.103229 [PubMed] [PMC]
- Hinder LM, Vincent AM, Hayes JM, McLean LL, Feldman EL (2013) Apolipoprotein E knockout as the basis for mouse models of dyslipidemia-induced neuropathy. Experimental Neurology 239: 102–110. https://doi.org/10.1016/j.expneurol.2012.10.002 [PubMed] [PMC]
- Holmberg O, Lenz T, Koch V, Alyagoob A, Utsch L, Rank A, Sabic E, Seguchi M, Xhepa E, Kufner S, Cassese S, Kastrati A, Marr C, Joner M, Nicol P (2021) Histopathology-based deep learning predicts atherosclerotic lesions in intravascular imaging. Frontiers in Cardiovascular Medicine 8: 779807. https://doi.org/10.3389/fcvm.2021.779807[PubMed] [PMC]
- Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, Tokgözoğlu L, Lewis EF (2019) Atherosclerosis. Nature Reviews Disease Primers 5(1): 56. https://doi.org/10.1038/s41572-019-0106-z [PubMed]
- Miano JM, Fisher EA, Majesky MW (2021) Fate and state of vascular smooth muscle cells in atherosclerosis. Circulation 143(21): 2110–2116. https://doi.org/10.1161/CIRCULATIONAHA.120.049922 [PubMed] [PMC]
- Mohanta S, Yin C, Weber C, Hu D, Habenicht AJ (2016) Aorta atherosclerosis lesion analysis in hyperlipidemic mice. Bio-Protocol Journal 6(11): e1833. https://doi.org/10.21769/bioprotoc.1833 [PubMed] [PMC]
- Noonan J, Cardoso L, Bobik A, Peter K (2025) Atherosclerotic plaque instability and rupture: recommended mouse models to empower clinically relevant discoveries, diagnostics, and therapeutics. Arteriosclerosis, Thrombosis and Vascular Biology 45(10): 1707–1714. https://doi.org/10.1161/ATVBAHA.125.321011 [PubMed] [PMC]
- Pedro-Botet J, Climent E, Benaiges D (2020) Atherosclerosis and inflammation: New therapeutic approaches. Medicina Clinica 155(6): 256–262. https://doi.org/10.1016/j.medcli.2020.04.024 [PubMed]
- Shcheblykin DV, Bolgov AA, Pokrovskii MV, Stepenko JV, Tsuverkalova JM, Shcheblykina OV, Golubinskaya PA, Korokina LV (2022) Endothelial dysfunction: developmental mechanisms and therapeutic strategies. Research Results in Pharmacology 8(4): 115–139. https://doi.org/10.3897/rrpharmacology.8.80376
- Sheng R, Li YN, Wang L, Jiang XH, Xiao TM, Zhang YH, Li SW, Wu YX, Xu Y, Xu YN, Si SY (2024) A novel small-molecule PCSK9 inhibitor E28362 ameliorates hyperlipidemia and atherosclerosis. Acta Pharmacologica Sinica 45(10): 2119–2133. https://doi.org/ 10.1038/s41401-024-01305-9 [PubMed] [PMC]
- Stadelmann VA, Boyd G, Guillot M, Bienvenu JG, Glaus C, Varela A (2021) Automatic quantification of atherosclerosis in contrast-enhanced microCT scans of mouse aortas ex vivo. International Journal of Biomedical Imaging 2021: 4998786. https://doi.org/10.1155/2021/4998786 [PubMed] [PMC]
- Stone PH, Libby P, Boden WE (2023) Fundamental pathobiology of coronary atherosclerosis and clinical implications for chronic ischemic heart disease management –the plaque hypothesis: a narrative review. JAMA Cardiology 8(2): 192–201. https://doi.org/10.1001/jamacardio.2022.3926 [PubMed] [PMC]

Author Contribution

- **Petr R. Lebedev**, junior researcher, Belgorod State National Research University, Belgorod, Russia; e-mail: artkelt98@yandex.ru; **ORCID ID:** https://orcid.org/0000-0001-9102-3360. The author was engaged in developing the concept, methodology and investigation.
- **Natalya V. Syrykh,** student, Belgorod State National Research University, Belgorod, Russia; e-mail: Nika150510@bk.ru; **ORCID ID:** https://orcid.org/0009-0004-3534-1590. The author was engaged in literature analysis and investigation.
- **Mikhail V. Pokrovskii**, Doctor Habil. of Medical Sciecnes, Professor, Department of Pharmacology and Clinical Pharmacology, e-mail: mpokrovsky@yandex.ru; **ORCID ID:**

https://orcid.org/0000-0002-1493-3376. The author set the research objectives, motivated the team, conducted a critical analysis of the material, and gave the final approval of the version of the manuscript.

Supplementary Material 1

Program code for quantification of atherosclerotic vascular lesion (ApoE−/−model)

Authors: Lebedev PR, Syrykh NV, Pokrovskii MV

Data type: pdf

Link: https://rrpharmacology.ru/index.php/journal/article/view/1078/702